49

CLAIMS

- Industrial process for preparing heterologous proteins in E. coli, in which E. coli
 bacteria modified with a suitable system for expressing heterologous proteins are seeded and cultured in a suitable culture medium, characterized in that the strain of E. coli is an E. coli strain W.
 - 2. Process according to claim 1,
- 10 characterized in that the strain W is the strain W deposited at the ATCC Inder the number 9637.
 - 3. Process according to claim 1, characterized in that the strain W is a derivative of the strain deposited at the ATCC under the number 9637, obtained by clonal selection or genetic manipulation.
- 4. Process according to one of claims 1 to 3, characterized in that the suitable culture medium is a culture medium which is suitable for the production of a high density of biomass and a high content of heterologous proteins produced.
 - 5. Process according to one of claims 1 to 4, characterized in that the culture medium comprises L-tryptophan.
 - 6. Process according to claim 5,
- 25 characterized in that the amount of L-tryptophan in the

REPLACEMENT SHEET (RULE 26)

50

culture medium is between 0.05 and 0.5 g/l, preferably between 0.1 and 0.3 g/l.

- 7. Process according to one of claims 1 to 6, characterized in that the culture medium comprises sucrose as the main carbon source.
- 8. Process according to claim 7, characterized in that the culture medium comprises substantially only sucrose as a carbon source.
- 9. Process according to either of claims 7
 10 and 8, characterized in that the amount of sucrose in the culture medium is between 0.1 and 300 g/l at the start of culturing, preferably between 0.5 and 200 g/l.
- 10. Process according to one of claims 1 to 9, characterized in that the suitable culture medium 15 also comprises a supplementary organic nitrogen source.
 - 11. Process according to claim 10, characterized in that the supplementary organic nitrogen source consists of protein extracts.
- and 10, characterized in that the protein extract has the following composition: (in g amino acids per 100 g of product) alanine between 10 and 4, aspartic [lacuna] between 11 and 4, glycine between 22 and 2.5 and lysine between 7 and 4.

- 13. Process according to one of claims 10 fo 12, characterized in that the supplementary organic nitrogen source consists of meat or potato peptones or proteins, more particularly the derivatives of potato 5 proteins.
 - 14. Process according to one of claims 1 to 13, characterized in that the suitable system for expressing heterologous proteins comprises a P_{trp} promoter.
- 15. Process according to claim 14, characterized in that the trp promoter comprises the nucleic acid sequence represented by sequence identifier no. 1 (SEQ ID NO 1).
- 16. Process according to one of claims 1 to
 15 15, characterized in that the heterologous protein is
 an enzyme.
 - 17. Process according to claim 16, characterized in that the enzyme is useful for the biocatalysis of chemical reactions.
- 20 18. Process according to claim 17, characterized in that the enzyme is a nitrilase.
 - 19. E. coli strain W, characterized in that it comprises a system for expressing heterologous proteins, in which the promoter is the P_{trp} promoter.

WO 99/64607

PCT/FR99/01343

52

20. Strain according to claim 19, characterized in that the P_{xrp} promoter comprises the nucleic acid sequence represented by sequence identifier no. 1 (SEQ ID NO 1).

WO)